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(54) Title: PEPTIDE INHIBITORS OF SELECTIN BINDING

(57) Abstract

The present invention provides novel peptides derived from portions of the sequence of amino acids 23-26 and 27-30 of P-selectin. The invention also provides pharmaceutical compositions comprising the peptides of the invention, and diagnostic and therapeutic methods utilizing the peptides and pharmaceutical compositions of the invention.

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PEPTIDE INHIBITORS OF SELECTIN BINDING

Background of the Invention

This invention relates to peptides which inhibit binding of selectins such as P-selectin, E-selectin and L-selectin.

The adherence of platelets and leukocytes to vascular surfaces is a critical component of the inflammatory response and is part of a complex series of reactions involving the simultaneous and interrelated activation of the complement, coagulation, and immune systems.

The complement proteins collectively play a leading role in the immune system, both in the identification and in the removal of foreign substances and immune complexes, as reviewed by Muller-Eberhard, H.J., Ann. Rev. Biochem. 57: 321-347 (1988). Central to the complement system are the C3 and C4 proteins, which when activated covalently attach to nearby targets, marking them for clearance. In order to help control this process, a remarkable family of soluble and membrane-bound regulatory proteins has evolved, each of which interacts with activated C3 and/or C4 derivatives. The coagulation and inflammatory pathways are regulated in a coordinate fashion in response to tissue damage. For example, in addition to becoming adhesive for leukocytes, activated endothelial cells express tissue factor on the cell surface and decrease their surface xpression of thrombomodulin, leading to a net facilitation of coagulation reactions on the cell surface. In some cases, a single

receptor can be involved in both inflammatory and coagulation processes.

Leukocyte adherence to vascular endothelium is a key initial step in migration of leukocytes to tissu s in response to microbial invasion. Although a class of inducible leukocyte receptors, the CD11-CD18 molecules, are thought to have some role in adherence to endothelium, mechanisms of equal or even greater importance for leukocyte adherence appear to be due to inducible changes in the endothelium itself.

Activated platelets have also been shown to interact with both neutrophils and monocytes *in vitro*. The interaction of platelets with monocytes may be mediated in part by the binding of thrombospondin to platelets and monocytes, although other mechanisms have not been excluded. The mechanisms for the binding of neutrophils to activated platelets are not well understood, except that it is known that divalent cations are required. In response to vascular injury, platelets are known to adhere to subendothelial surfaces, become activated, and support coagulation. Platelets and other cells may also play an important role in the recruitment of leukocytes into the wound in order to contain microbial invasion.

Endothelium exposed to "rapid" activators such as thrombin and histamine becomes adhesive for neutrophils within two to ten minutes, while endothelium exposed to cytokines such as tumor necrosis factor and interleukin-1 becomes adhesive after one to six hours. The rapid endothelial-dependent leukocyte adhesion has been associated with expression of the lipid mediator platelet activating factor (PAF) on the cell surface, and presumably, the appearance of other endothelial surface receptors. The slower cytokine-inducible endothelial adhesion for leukocytes is mediated, at least in part, by E-selectin that is synthesized by endothelial cells after exposure to cytokines and then transported to the cell surface, wher it binds neutrophils. The isolation, characterization and cloning of

E-selectin or ELAM-1 is reviewed by Bevilacqua, et al., in Scienc 243, 1160-1165 (1989). L-s l ctin, a peripheral lymph node homing receptor, also called "the murine Mel 14 antigen", "Leu 8", the "Leu 8 antigen" and "LAM-1", is another structure on neutrophils, monocytes, and lymphocytes that binds lymphocytes to high endothelial venules in peripheral lymph nodes. The characterization and cloning of the protein is reviewed by Lasky, et al., Cell 56, 1045-1055 (1989) (mouse) and Tedder, et al., J. Exp. Med. 170, 123-133 (1989).

P-selectin, also known as GMP-140 (granule membrane protein 140), or PADGEM, is a cysteine-rich and heavily glycosylated integral membrane glycoprotein with an apparent molecular weight of 140,000 as assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). P-selectin was first purified from human platelets by McEver and Martin, J. Biol. Chem. 259: 9799-9804 (1984). The protein is present in alpha granules of resting platelets but is rapidly redistributed to the plasma membrane following platelet activation, as reported by Stenberg, et al., (1985). The presence of P-selectin in endothelial cells and its biosynthesis by these cells was reported by McEver, et al., Blood 70(5) Suppl. 1:355a, Abstract No. 1274 (1987). In endothelial cells, P-selectin is found in storage granules known as the Weibel-Palade bodies. (McEver, et al. J. Clin. Invest. 84: 92-99 (1989) and Hattori, et al., J. Biol. Chem. 264: 7768-7771 (1989)). P-selectin (called GMP-140 or PADGEM) has also been reported to mediate the interaction of activated platelets with neutrophils and monocytes by Larsen, et al., in Cell 59, 305-312 (October 1989) and Hamburger and McEver, Blood 75: 550-554 (1990).

The cDNA-derived amino acid sequence, reported by Johnston, et al., in Cell 56, 1033-1044 (March 24 1989), and in U.S. Serial No. 07/320,408 filed March 8, 1989, indicates that it contains a number f modular domains that are likely to fold independ ntly. Beginning at the N-terminus, these include a "lectin" domain, an "EGF" domain, nine tandem

consensus repeats similar to those in complement binding proteins, a transmembrane domain (except in a soluble form that app ars to result from differential splicing), and a cytoplasmic tail.

When platelets or endothelial cells are activated by mediators such as thrombin, the membranes of the storage granules fuse with the plasma membrane, the soluble contents of the granules are released to the external environment, and membrane bound P-selectin is presented within seconds on the cell surface. The rapid redistribution of P-selectin to the surface of platelets and endothelial cells as a result of activation suggested that this glycoprotein could play an important role at sites of inflammation or vascular disruption.

This important role has been confirmed by the observation that P-selectin is a receptor for neutrophils (Geng et al., Nature 343:757-760 (1990); Hamburger and McEver, Blood 75:550-554 (1990)), monocytes (Larsen, et al. Cell 59:305-312 (1989)); Moore, et al., J. Cell Biol. 112:491-499 (1991)), and perhaps a subset of lymphocytes (Moore, et al. J. Cell Biol. 112:491-499 (1991)). Thus, GMP-140 can serve as a receptor for leukocytes following its rapid mobilization to the surfaces of platelets and endothelial cells stimulated with agonists such as thrombin. This role in leukocyte recruitment may be important in hemostatic and inflammatory processes in both physiologic and pathologic states.

Peptides derived from P-selectin are described in U.S. Serial No. 07/554,199 entitled "Functionally Active Selectin-Derived Peptides" filed July 17, 1990 by Rodger P. McEver that are useful in diagnostics and in modulating the hemostatic and inflammatory responses in a patient wherein a therapeutically effective amount of a peptide capable of blocking leukocyte recognition of P-selectin is administered to the patient. U.S. Serial No. 07/554,199 filed July 17, 1990 also discloses that peptide sequences within the lectin domain of P-selectin, having homology with the lectin domains

of other proteins, especially E-s lectin and the L-sel ctin, selectively inhibit neutrophil adhesion to purified P-selectin, and can therefore be used in diagnostic assays of patients and diseases characterized by altered binding by these molecules, in screening assays for compounds altering this binding, and in clinical applications to inhibit or modulate interactions of leukocytes with platelets or endothelial cells involving coagulation and/or inflammatory processes.

E-selectin, L-selectin, and P-selectin have been termed "selectins", based on their related structure and function. E-selectin is not present in unstimulated endothelium. However, when endothelium is exposed to cytokines such as tumor necrosis factor of interleukin-1, the gene for E-selectin is transcribed, producing RNA which in turn is translated into protein. The result is that E-selectin is expressed on the surface of endothelial cells one to four hours after exposure to cytokines, as reported by Bevilacqua et al., Proc.Natl.Acad.Sci.USA 84: 9238-9242 (1987) (in contrast to P-selectin, which is stored in granules and presented on the cell surface within seconds after activation). E-selectin has been shown to mediate the adherence of neutrophils to cytokine-treated endothelium and thus appears to be important in allowing leukocytes to migrate across cytokine-stimulated endothelium into tissues. The cDNA-derived primary structure of E-selectin indicates that it contains a "lectin" domain, an EGF domain, and six (instead of the nine in P-selectin) repeats similar to those of complement-regulatory proteins, a transmembrane domain, and a short cytoplasmic tail. There is extensive sequence homology between P-selectin and E-selectin throughout both proteins, but the similarity is particularly striking in the lectin and EGF domains.

Homing receptors ar lymphocyte surface structures that allow lymphocytes to bind to specialized endothelial cells in lymphatic tissues, termed high endothelial cells or high endothelial venules (reviewed by Yednock and Rose,

Advances in Immunology, vol. 44, F.I. Dixon, d., 313-378 (Academic Pr ss, N w York 1989). This binding allows lymphocytes to migrate across the endothelium into the lymphatic tissues wh re they are exposed to process d antigens. The lymphocytes then re-enter the blood through the lymphatic system. L-selectin, a lymphocyte homing receptor, contains a lectin domain, an EGF domain, two complement-binding repeats, a transmembrane domain, and a short cytoplasmic tail. L-selectin also shares extensive sequence homology with P-selectin, particularly in the lectin and EGF domains.

Based on a comparison of the lectin domains between P-selectin, E-selectin, and L-selectin, it may be possible to select those peptides inhibiting binding of neutrophils to P-selectin which will inhibit binding of E-selectin, L-selectin, and other homologous selectins, to components of the inflammatory process, or, conversely, which will inhibit only P-selectin binding.

The *in vivo* significance of platelet-leukocyte interactions has not been studied carefully. However, in response to vascular injury, platelets are known to adhere to subendothelial surfaces, become activated, and support coagulation. Platelets and other cells may also play an important role in the recruitment of leukocytes into the wound in order to contain microbial invasion. Conversely, leukocytes may recruit platelets into tissues at sites of inflammation, as reported by Issekutz, et al., Lab. Invest. 49:716 (1983).

The coagulation and inflammatory pathways are regulated in a coordinate fashion in response to tissue damage. For example, in addition to becoming adhesive for leukocytes, activated endothelial cells express tissue factor on the cell surface and decrease their surface expression of thrombomodulin, leading to a net facilitation of coagulation reactions on the cell surface. In some cases, a single receptor can be involv d in both inflammatory and coagulation processes.

Proteins involved in the hemostatic and inflammatory pathways are of interest for diagnostic purposes and treatment of human disorders. However, there are many problems using proteins therapeutically. Proteins are usually expensive to produce in quantities sufficient for administration to a patient. Moreover, there can be a reaction against the protein after it has been administered more than once to the patient. It is therefore desirable to develop peptides having the same, or better, activity as the protein, which are inexpensive to synthesize, reproducible and relatively innocuous.

It is preferable to develop peptides which can be prepared synthetically, having activity at least equal to, or greater than, the peptides derived from the protein itself.

It is therefore an object of the present invention to provide peptides interacting with cells recognized by selectins, including P-selectin, E-selectin, and L-selectin.

It is another object of the present invention to provide methods for using these peptides to inhibit leukocyte adhesion to endothelium or to platelets.

It is a further object of the present invention to provide methods for using these peptides to modulate the immune response and the hemostatic pathway.

It is yet another object of the present invention to provide peptides for use in diagnostic assays relating to P-selectin, E-selectin and L-selectin.

Summary of the Inventi n

This invention relates to novel peptides selected from formulas (I) and (II):

$$R^1\text{-W-A-B-C-D-X-}R^2$$

(I)

and

$$R^1\text{-Y-E-F-G-H-Z-}R^2$$

(II)

or pharmaceutically acceptable salts thereof, where:

W and Y are an N-terminus amino acid linear sequence of from zero to 10 amino acids, and R^1 is a moiety attached to the terminal α amino group of W or Y, or the terminal α-amino group of A or E if W or Y respectively is zero;

X and Z are a C-terminus amino acid linear sequence of from zero to 10 amino acids, and R^2 is a moiety attached to the carboxyl carbon of X or Z ($C(O)R^2$) or the carboxyl carbon of D or H if X or Z respectively is zero;

A is an amino acid selected from the group consisting of D- or L-tyrosine, D- or L-phenylalanine, D- or L-lysine, D- or L-glutamic acid, D- or L- arginine, D- or L-cysteine, D- or L-O-R^3-tyrosine, D- or L-N^α-R^3-tyrosine, D- or L-4-amino phenylalanine, D- or L-R^4-phenylalanine, D- or L-naphthylalanine, D- or L-pyridylalanine or D-L-tetrahydroisoquiniline carboxylic acid, where R^3 is lower alkyl or aryl, and R^4 is halogen;

B is an amino acid selected from the group consisting of D- or L-threonine, D- or L-lysine, D- or L-glutamic acid, D- or L-cysteine or glycine;

C is an amino acid selected from the group consisting of D- or L-aspartic acid, D-or L-histidine, D- or L-glutamic acid, D- or L-asparagine, D- or L-glutamine, D- or L-alanine, D- or L-phenylalanine, D- or L-lysine or glycine;

D is an amino acid selected from the group consisting of D- or L-leucine, D- or L-isol ucine, D- or L-alanine, D- or L-valine, D- or L-alleisoleucine, glycine, D-

or L-glutamic acid, D- or L-aspartic acid, D- or L-asparagine, D- or L-glutamine or D- or L-threonine;

provided that X is not J-K-L-Z′, where J is an amino acid selected from the group consisting of L-leucine, D-glutamic acid, D- or L-isoleucine, D- or L-alanine, D- or L-valine, glycine, D- or L-glutamic acid, D- or L-aspartic acid, D- or L-asparagine, and D- or L- glutamine; D- or L-threonine; and D- or L-alloisoleucine; K is D- or L-leucine, D- or L-isoleucine, D- or L-alanine, D- or L valine, glycine, D- or L-glutamic acid, D- or L-aspartic acid, D- or L-asparagine, D- or L-glutamine, D- or L threonine and D- or L-alloisoleucine; L is D- or L-glutamine, D- or L-glutamic acid, and D- or L-asparagine; and Z′ is a linear chain of from 0 to 7 amino acids;

E is an amino acid selected from the group consisting of D- or L-leucine, D- or L-isoleucine, D- or L-alanine, D- or L-valine, D- or L-alloisoleucine, glycine, D- or L-glutamic acid, D- or L-aspartic acid, D- or L-asparagine, D- or L-glutamine or D- or L-threonine;

F is an amino acid selected from the group consisting of D- or L-leucine, D- or L-isoleucine, D- or L-alanine, D- or L-valine, D- or L-alloisoleucine, glycine, D- or L-glutamic acid, D- or L-aspartic acid, D- or L-asparagine, D- or L-glutamine or D- or L-threonine;

G is an amino acid selected from the group consisting of D- or L-leucine, D- or L-isoleucine, D- or L-alanine, D- or L-valine, D- or L-alloisoleucine, glycine, D- or L-glutamic acid, D- or L-aspartic acid, D- or L-asparagine, D- or L-glutamine or D- or L-threonine;

H is an amino acid selected from the group consisting of glutamine, D-glutamine, glutamic acid, D-glutamic acid, asparagine or D-asparagine;

provided that Y is not M-N-P-Z″, where M is D- or L-tyrosine, D- or L-phenylalanine, D- or L-lysine, D- or L-glutamic acid, D- or L-arginine, D- or L- cyst ine, D- or L-4-amino ph nylalanine, D- or L-naphthylalanine, D- or l-pyridylalanine, D- or L-tetrahydroisoquiniline, D- or L-O-R^3-

tyrosine, D- or L-N^2-R^3-tyrosine, D- or L-R^4-phenylalanine, where R^3 is lower alkyl or aryl, and R^4 is halogen; N is an amino acid sel cted from the group consisting of D- or L-threonine, D- or L-lysine, D- or L-glutamine acid, D- or L-cysteine, and glycine; P is an amino acid selected from the group consisting of D- or L-aspartic acid, D- or L-histidine, D- or L-glutamic acid, D- or L-asparagine, D- or L-glutamine, D or L-alanine, D- or L-phenylalanine, D- or L-lysine and glycine; and Z″ is a linear amino acid chain of from 0 to 7 amino acids.

R^1 is hydrogen (signifying a free N-terminal group), lower alkyl, aryl, formyl, alkanoyl, aroyl, alkyloxycarbonyl or arroyloxycarbonyl;

R^2 is OH (signifying a free C-terminal carboxylic acid), OR^3, signifying ester, where R^3 is lower alkyl or aryl or R^2 is NR^5R^6 where R^5 and R^6 are each selected independently from hydrogen, lower alkyl, aryl or cyclic alkyl;

Peptides of Formula I and II have as their core region, respectively, portions of the 23-26 and 27-30 amino acid sequences of P-selectin, with residue 1 defined as the N-terminus of the mature protein after the cleavage of the signal peptide.

Tests indicate that the peptides of Formulas I and II inhibit the binding of neutrophils to P-selectin in concentrations of peptide ranging from about 50 to about 1500 μm. Tests also indicate that alterations within the core sequence, as well as N-terminal and C-terminal flanking regions, do not result in loss of biological activity.

This invention relates not only to the novel peptides of Formulas I and II, but also to pharmaceutical compositions comprising them, to diagnostic and therapeutic methods utilizing them, and to methods of preparing them.

Brief D scripti n f the Drawings

Figur I shows peptides of Formula I to inhibit the adhesion of human neutrophils to purified human P-selectin.

Detailed Description of the Invention

Preferred peptides of this invention are those of Formulas I and II wherein, together or independently: R^1 is hydrogen or acetyl (Ac); R^2 is OH or NH_2; A is Tyr, D-Tyr, Arg or D-Arg; C is Asp, D-Asp, His or D-His.

Specifically preferred peptides include the following:

Arg-Tyr-Thr-Asp-Leu-Val-Ala-Ile-NH_2 (SEQ ID NO: 1)

Asn-Arg-Tyr-Thr-Asp-Leu-Val-Ala-Ile-NH_2 (SEQ ID NO: 2)

Asn-Arg-Tyr-Thr-Asp-Leu-Val-Ala-NH_2 (SEQ ID NO: 3)

Tyr-Thr-Asp-Leu-Val-Ala-Ile-NH_2 (SEQ ID NO: 4)

Asn-Arg-Tyr-Thr-Asp-Leu-Val-NH_2 (SEQ ID NO: 5)

Asp-Leu-Val-Ala-Ile-Gln-Asn-Lys-Asn-Glu-NH_2 (SEQ ID NO: 6)

Thr-Asp-Leu-Val-Ala-Ile-Gln-Asn-Lys-Asn-Glu-NH_2 (SEQ ID NO: 7)

Leu-Val-Ala-Ile-Gln-Asn-Lys-Asn-Glu-NH_2 (SEQ ID NO: 8)

Thr-Asp-Leu-Val-Ala-Ile-Gln-NH_2 (SEQ ID NO: 9)

As used herein, the term "alkyl" includes branched, straight-chain, and cyclic saturated hydrocarbons. The term "lower alkyl" means an alkyl having from one to six carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, pentyl, isopentyl, neopentyl, cyclopentylmethyl and hexyl. The term "alkanoyl" means

$$R^7-\overset{\displaystyle O}{\overset{\|}{C}}-$$

wherein R^7 is a alkyl group.

The term "aroyl" means

$$R^{8}-\overset{\overset{\displaystyle O}{\|}}{C}-$$

wherein R^8 is an aryl group. The term "aryl" means an aromatic or heteroaromatic structure having between one and three rings, which may or may not be ring fused structures, and are optimally substituted with halogens, carbons, or other heteroatoms such as nitrogen (N), sulfur (S), phosphorus (P), and boron (B).

The term alkoxycarbonyl means

$$R^{9}-O-\overset{\overset{\displaystyle O}{\|}}{C}-O-$$

wherein R^9 is a lower alkyl group.

The term aryloxycarbonyl means

$$R^{10}-O-\overset{\overset{\displaystyle O}{\|}}{C}-O-$$

wherein R^{10} is an aryl and arylmethyl group.

Halogen refers to fluorine, chlorine, bromine or iodine.

The term "terminal α-amino group of X" refers to the α-amino group of the N-terminal amino acid of X.

The peptides of Formula I and II can be used in the form of the free peptide or a pharmaceutically acceptable salt. Amine salts can be prepared by treating the peptide with an acid according to known methods. Suitable acids include inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid, anthranilic acid, cinnamic acid, naphthalenesulfonic acid, and sulfanilic acid.

Carboxylic acid groups in the peptide can be converted to a salt by treating the peptide with a base according to known methods. Suitable bases include inorganic bases such as sodium hydroxide, ammonium hydroxide, and

potassium hydroxide, and organic bases such as mono-, di-, and tri-alkyl and aryl amines (e.g., triethylamine, diisopropylamine, methylamine, and dimethylamine and optionally substituted mono-, di, and tri-ethanolamines.

As referred to herein, the amino acid components of the peptides and certain materials used in their preparation are identified by abbreviations for convenience. These abbreviations are as follows:

Amino Acid	Abbreviations	
L-alanine	Ala	A
D-alanine	D-Ala	a
L-arginine	Arg	R
D-arginine	D-Arg	r
D-asparagine	D-Asn	N
L-asparagine	L-Asn	n
L-aspartic acid	Asp	D
D-aspartic acid	D-Asp	d
L-cysteine	Cys	C
D-cysteine	D-Cys	c
L-glutamic acid	Glu	E
D-glutamic acid	D-Glu	e
L-glutamine	Gln	Q
D-glutamine	D-Gln	q
glycine	Gly	G
L-histidine	His	H
D-histidine	D-His	h
L-isolelucine	Ile	I
D-isolelucine	D-Ile	i
L-leucine	Leu	L
D-leucine	D-Leu	l
L-lysine	Lys	K
D-lysine	D-Lys	k
L-phenylalanine	Phe	F
D-phenylalanine	D-Phe	f
L-proline	Pro	P
D-proline	D-Pro	p
L-pyroglutamic acid	pGlu	
D-pyroglutamic acid	DpGlu	
L-serine	L-Ser	S
D-serine	D-Ser	s
L-threonine	L-Thr	T
D-threonine	D-Thr	t
L-tyrosine	L-Tyr	Y
D-tyrosine	D-Tyr	y
L-tryptophan	Trp	W
D-tryptophan	D-Trp	w
L-valine	Val	V
D-valine	D-Val	v
L-alloisolucine	All	
D-alloisolucine	D-Allo	
α,α,α-trifluoro - m-toluidino-nicotinyl	Nif	

Reagents	Abbreviations
Trifluoroacetic acid	TFA
M thyl n chlorid	CH_2Cl_2
N,N-Diisopropylethylamine	DIEA
N-Methylpyrrolidone	NMP
1-Hydroxybenzotriazole	HOBT
Dimethylsulfoxide	DMSO
Acetic anhydride	Ac_2O

Amino acids preceeded by L- or D- refer, respectively, to the L- or D- enantiomer of the amino acid, whereas amino acids not preceeded by L- or D- refer to the L- enantiomer.

Methods of Preparation of Peptides

The peptides can generally be prepared following known techniques, as described, for example, in the cited publications, the teachings of which are specifically incorporated herein. In a preferred method, the peptides are prepared following the solid-phase synthetic technique initially described by Merrifield in J.Amer.Chem.Soc., 85, 2149-2154 (1963). Other techniques may be found, for example, in M. Bodanszky, et al, Peptide Synthesis, second edition, (John Wiley & Sons, 1976), as well as in other reference works known to those skilled in the art.

Appropriate protective groups usable in such syntheses and their abbreviations will be found in the above text, as well as in J.F.W. McOmie, Protective Groups in Organic Chemistry, (Plenum Press, New York, 1973). The common protective groups used herein are t-butyloxycarbonyl (Boc), fluorenylmethoxycarboyl (FMOC), benzyl (Bzl), tosyl (Tos), o-bromo-phenylmethoxycarbonyl (BrCBZ), phenylmethoxycarbonyl (CBZ), 2-chloro-phenylmethoxycarbonyl (2-Cl-CBZ), 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr), trityl (Trt), formyl (CHO), and tertiary butyl (t-Bu).

General synthetic procedures for the synthesis of peptides of Formulas I and II by solid phase methodology are as follows:

A. General Synthetic Proc dures For S lid Phas Peptide Synth sis Using N^{α}-B c Pr t cti n

		REPETITIONS	TIME
1.	25% TFA in CH_2Cl_2	1	3 min.
2.	50% TFA in CH_2Cl_2	1	16 min.
3.	CH_2Cl_2	5	3 min.
4.	5% DIEA in NMP	2	4 min.
5.	NMP	6	5 min.
6.	Coupling step	1	57 min.
	a. Preformed BOC-Amino Acid-HOBT active ester in NMP		37 min.
	b. DMSO		16 min.
	c. DIEA		5 min.
7.	10% Ac_2O, 5% DIEA in NMP	1	9 min.
8.	CH_2Cl_2	5	3 min.

B. General Synthetic Procedure For Solid Phase Peptide Synthesis Using N^{α}-FMOC Protection

		REPETITIONS	TIME
1.	20% piperidine in NMP	1	3 min.
2.	20% piperidine in NMP	1	15 min.
3.	NMP	6	9 min.
4.	Coupling Preformed FMOC-Amino Acid-HOBT active ester in NMP or FMOC-amino acid and HOBT in NMP followed by the addition DIC in NMP.	1	71 min.
5.	NMP	6	7 min.

N-terminal acetylation on the deprotected N^{α}-amino group of peptides synthesized using either Boc or FMOC strategies is accomplished with 10% Ac_2O and 5% DIEA in NMP, followed by washing of the peptide resin with NMP and/or CH_2Cl_2.

The peptides can also be prepared using standard genetic engineering techniques known to those skilled in the

art. For example, the peptide can be pr duced nzymatically by inserting nucleic acid encoding the peptide into an expression vector, expressing the DNA, and translating the DNA into the peptide in the presence of the required amino acids. The peptide is then purified using chromatographic or electrophoretic techniques, or by means of a carrier protein which can be fused to, and subsequently cleaved from, the peptide by inserting into the expression vector in phase with the peptide encoding sequence a nucleic acid sequence encoding the carrier protein. The fusion protein-peptide may be isolated using chromatographic, electrophoretic or immunological techniques (such as binding to a resin via an antibody to the carrier protein). The peptide can be cleaved using chemical methodology or enzymatically, as by, for example, hydrolases.

Peptides of Formula I can also be prepared using solution methods, by either stepwise or fragment condensations. An appropriately alpha amino-protected amino acid is coupled to an appropriately alpha carboxyl protected amino acid (such protection may not be required depending on the coupling method chosen) using diimides, symmetrical or unsymmetrical anhydrides, BOP, or other coupling reagents or techniques known to those skilled in the art. These techniques may be either or enzymatic. The alpha amino and/or alpha carboxyl protecting groups are removed and the next suitably protected amino acid or block of amino acids are coupled to extend the growing peptide. Various combinations of protecting groups and of chemical and/or enzymatic techniques and assembly strategies can be used in each synthesis.

Methods of Preparation of Pharmaceutical Compositions

Pharmaceutical compositions of this invention comprise a pharmaceutically acceptable carrier or diluent and an effective quantity of one or more of the peptides of Formulas I or II or an acid or base salt ther of. The carrier or diluent may take a wide variety of forms depending on the

form of preparation desired for administration, e.g., sublingual, rectal, nasal, oral, or parenteral.

In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, for example, waters, oils, alcohols, flavoring agents, preservatives, and coloring agents, to make an oral liquid preparation (e.g., suspension, elixir, or solution) or with carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, and disintegrating agents, to make an oral solid preparation (e.g., powder, capsule, or tablet).

Controlled release forms or enhancers to increase bioavailability may also be used. Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are employed. If desired, tablets may be sugar coated or enteric coated by standard techniques.

For parenteral products, the carrier will usually be sterile water, although other ingredients to aid solubility or as preservatives may be included. Injectable suspensions may also be prepared, in which case appropriate liquid carriers and suspending agents can be employed.

The peptides can also be administered locally at a wound or inflammatory site by topical application of a solution or cream.

Alternatively, the peptide may be administered in liposomes or microspheres (or microparticles). Methods for preparing liposomes and microspheres for administration to a patient are known to those skilled in the art. U.S. Patent No. 4,789,734 describes methods for encapsulating biological materials in liposomes. Essentially, the material is dissolved in an aqueous solution, the appropriate phospholipids and lipids added, along with surfactants if required, and th material dialyzed or sonicated, as necessary. A review of known methods is by G. Gregoriadis, Chapt r 14, "Liposomes", Drug Carriers in Biology and Medicine, pp. 287-341 (Academic Press, 1979). Microspheres

formed of polymers or proteins are well known to those skill d in the art, and can be tailored for passage through the gastrointestinal tract directly into the bloodstream. Alternatively, the peptide can be incorporated and the microspheres, or composite of microspheres, implanted for slow release over a period of time, ranging from days to months. See, for example, U.S. Patents Nos. 4,906,474, 4,925,673 and 3,625,214.

The peptides are generally active when administered parenterally in amounts above about 1 μg/kg body weight. The peptides are generally active when administered parenterally in amounts above about 1 μg/kg body weight. Effective doses by other routes of administration are generally those which result in similar blood level to i.v. doses above about 1 μg/Kg. For treatment to prevent organ injury in cases involving reperfusion, the peptides may be administered parenterally in amounts from about 0.01 to about 10 mg/kg body weight. Generally, the same range of dosage amounts may be used in treatment of other diseases or of conditions where inflammation is to be reduced. This dosage will be dependent, in part, on whether one or more peptides are administered. A synergistic effect may be seen with combinations of peptides from different, or overlapping, regions of the lectin domain, or in combination with peptides derived form the EGF domain of P-selectin. For treatment to prevent organ injury in cases involving reperfusion, the peptides may be administered parenterally in amounts from about 0.01 to about 10 mg/kg body weight. Generally, the same range of dosage amounts may be used in treatment of other diseases or of conditions where inflammation is to be reduced. This dosage will be dependent, in part, on whether one or more peptides are administered. A synergistic effect may be seen with combinations of peptides from different, or overlapping, regions of the lectin domain, or in combination with peptides derived form the EGF domain of P-selectin.

M thods f r Dem nstrating Binding

P ptid s that are biologically active are thos which inhibit binding of neutrophils, monocytes, subsets of lymphocytes or other cells to P-selectin, or which inhibit leukocyte adhesion to endothelium that is mediated by ELAM-1 and/or the homing receptor.

Peptides can be screened for their ability to inhibit adhesion to cells, for example, neutrophil adhesion to purified P-selectin immobilized on plastic wells, using the assay described by Geng, et al., Nature 343, 757-760 (1990).

Human neutrophils are isolated from heparinized whole blood by density gradient centrifugation on Mono-Poly resolving media, Flow Laboratories. Neutrophil suspensions are greater than 98% pure and greater than 95% viable by trypan blue exclusion. For adhesion assays, neutrophils are suspended at a concentration of 2×10^6 cells/mL in Hanks' balanced salt solution containing 1.26 mM Ca^{2+} and 0.81 mM Mg^{2+} (HBSS, Gibco) with g mg/mL human serum albumin (HBSS/HSA). Adhesion assays are conducted in triplicate in 96-well microtiter plates, Corning, incubated at 4°C overnight with 50 microliters of various protein solutions.

P-selectin is isolated from human platelet lysates by immunoaffinity chromatography on antibody S12-Sepharose™ and ion-exchange chromatography on a Mono-Q™ column (FLPC, Pharmacia Fine Chemicals), as follows.

Outdated human platelet packs (100 units) obtained from a blood bank and stored at 4°C are pooled, adjusted to 5mM EDTA at pH 7.5, centrifuged at 4,000 rpm for 30 minutes in 1 liter bottles, then washed three times with 1 liter of 0.1 M NaCl, 20 mM Tris pH 7.5 (TBS), 5 mM EDTA, 5 mM benzamidine.

The pellets are then resuspended in a minimum amount of wash buffer and made 1mM in DIFP, then frozen in 50 mL screwtop tubes at -80°C. The frozen platelets are thawed and resusp nd d in 50 mL TBS, 5 mM benzamidine, 5 mM EDTA pH 7.5, 100 M leupeptin. Th suspension is frozen and thaw d two times in a dry ice-acetone bath using a 600 mL lyophilizing

flask, then homogenized in a glass/teflon mortar and pestle and made 1 mM in DIFP. The NaCl c ncentration is adjusted to 0.5 M with a stock solution of 4 M NaCl. After stirring the susp nsion at 4°C, it is centrifuged in polycarbonate tubes at 33,000 rpm for 60 minutes at 4°C. The supernatant (0.5 M NaCl wash) is removed and saved; this supernatant contains the soluble form of P-selectin. Care is taken not to remove the top part of the pellet with the supernatant. The pellets are then homogenized in extraction buffer (TBS, 5 mM benzamidine, 5 mM EDTA, pH 7.5, 100 μM leupeptin, 2% Triton X-100). After centrifugation at 19,500 rpm for 25 minutes at 4°C, the supernatant is removed. The extraction procedure is repeated with the pellet and the supernatant is combined with the first supernatant. The combined extracts, which contain the membrane form of P-selectin, are adjusted to 0.5 M NaCl.

The soluble fraction (0.5 M NaCl wash) and the membrane extract (also adjusted to 0.5 M NaCl) are absorbed with separate pools of the monoclonal antibody S12 (directed to P-selectin) previously coupled to Affigel (Biorad) at 5 mg/mL for 2 hours at 4°C. After letting the resins settle, the supernatants are removed. The S12 Affigel containing bound GMP-140 is then loaded into a column and washed overnight at 4°C with 400 mL of 0.5 M NaCl, 20 mM Tris pH 7.5, 0.01% Lubrol PX.

Bound P-selectin is eluted from the S12 Affigel with 100 mL of 80% ethylene glycol, 1 mM MES pH 6.0, 0.01% Lubrol PX. Peak fractions with absorbance at 280 nm are pooled. Eluates are dialyzed against TBS with 0.05% Lubrol, then applied to a Mono Q column (FPLC from Pharmacia). The concentrated protein is step eluted with 2 M NaCl, 20 mM Tris pH 7.5 (plus 0.05% Lubrol PX for the membrane fraction). Peak fractions are dialyzed into TBS pH 7.5 (plus 0.05% Lubrol PX for the membrane fraction).

P-selectin is plated at 5 micrograms/mL and the control proteins: human serum albumin (Alb), platelet glycoprotein IIb/IIIa (IIb), von Willebrand factor (vWF), fibrinogen (FIB), thrombomodulin (TM), gelatin (GEL) or human

serum (HS), are added at 50 micrograms/mL. All wells are blocked for 2 hours at 22°C with 300 microliters HBSS containing 10 mg/mL HSA, then washed three times with HBSS containing 0.1% Tween-20 and once with HBSS. Cells (2 x 10^5 per well) are added to the wells and incubated at 22°C for 20 minutes. The wells are then filled with HBSS/HSA, sealed with acetate tape (Dynatech), and centrifuged inverted at 150 g for 5 minutes. After discarding nonadherent cells and supernates, the contents of each well are solubilized with 200 microliters 0.5% hexadecyltrimethylammonium bromide, Sigma, in 50 mM potassium phosphate, pH. 6.0, and assayed for myeloperoxidase activity, Ley, et al., Blood 73, 1324-1330 (1989). The number of cells bound is derived from a standard curve of myeloperoxidase activity versus numbers of cells. Under all assay conditions, the cells release less than 5% of total myeloperoxidase and lactate dehydrogenase. Inhibition is read as a lower percent adhesion, so that a value of 5% means that 95% of the specific adhesion was inhibited.

Clinical Applications

Since the selectins have several functions related to leukocyte adherence, inflammation, and coagulation, compounds which interfere with binding of P-selectin, E-selectin or L-selectin can be used to modulate these responses.

For example, the peptides can be used to competitively inhibit leukocyte adherence by competitively binding to P-selectin receptors on the surface of leukocytes. This kind of therapy would be particularly useful in acute situations where effective, but transient, inhibition of leukocyte-mediated inflammation is desirable. Chronic therapy by infusion of the peptides may also be feasible in some circumstances.

An inflammatory response may cause damage to the host if unchecked, because leukocytes release many toxic molecules that can damage normal tissues. These molecules include proteolytic enzymes and free radicals. Examples of pathological situations in which leukocytes can cause tissue damage include injury from ischemia and reperfusion,

bacterial sepsis and disseminated intravascular coagulation, adult respiratory distress syndrome, tumor metastasis, rheumatoid arthritis and atherosclerosis.

Reperfusion injury is a maj r problem in clinical cardiology. Therapeutic agents that reduce leukocyte adherence in ischemic myocardium can significantly enhance the therapeutic efficacy of thrombolytic agents. Thrombolytic therapy with agents such as tissue plasminogen activator or streptokinase can relieve coronary artery obstruction in many patients with severe myocardial ischemia prior to irreversible myocardial cell death. However, many such patients still suffer myocardial neurosis despite restoration of blood flow. This "reperfusion injury" is known to be associated with adherence of leukocytes to vascular endothelium in the ischemic zone, presumably in part because of activation of platelets and endothelium by thrombin and cytokines that makes them adhesive for leukocytes (Romson et al., Circulation 67: 1016-1023 (1983)). These adherent leukocytes can migrate through the endothelium and destroy ischemic myocardium just as it is being rescued by restoration of blood flow.

There are a number of other common clinical disorders in which ischemia and reperfusion results in organ injury mediated by adherence of leukocytes to vascular surfaces, including strokes; mesenteric and peripheral vascular disease; organ transplantation; and circulatory shock (in this case many organs might be damaged following restoration of blood flow).

Bacterial sepsis and disseminated intravascular coagulation often exist concurrently in critically ill patients. They are associated with generation of thrombin, cytokines, and other inflammatory mediators, activation of platelets and endothelium, and adherence of leukocytes and aggregation of platelets throughout the vascular system. Leukocyte-dependent organ damage is an important feature of th s conditions.

Adult respiratory distress syndrom is a devastating pulmonary disord r occurring in patients with sepsis or following trauma, which is associated with widespread adherence and aggregation of leukocytes in the pulmonary circulation. This leads to extravasation of large amounts of plasma into the lungs and destruction of lung tissue, both mediated in large part by leukocyte products.

Two related pulmonary disorders that are often fatal are in immunosuppressed patients undergoing allogeneic bone marrow transplantation and in cancer patients suffering from complications that arise from generalized vascular leakage resulting from treatment with interleukin-2 treated LAK cells (lymphokine-activated lymphocytes). LAK cells are known to adhere to vascular walls and release products that are presumably toxic to endothelium. Although the mechanism by which LAK cells adhere to endothelium is now known, such cells could potentially release molecules that activate endothelium and then bind to endothelium by mechanisms similar to those operative in neutrophils.

Tumor cells from many malignancies (including carcinomas, lymphomas, and sarcomas) can metastasize to distant sites through the vasculature. The mechanisms for adhesion of tumor cells to endothelium and their subsequent migration are not well understood, but may be similar to those of leukocytes in at least some cases. The association of platelets with metastasizing tumor cells has been well described, suggesting a role for platelets in the spread of some cancers. Recently, it was reported that P-selectin binds to tumor cells in a variety of human carcinoma tissue sections (colon, lung, and breast), and that P-selectin binds to the cell surface of a number of cell lines derived from various carcinomas, but not from melanomas. Aruggo, A., et al., Proc. Natl. Acad. Sci. USA, 89, 2292-2296 (1992). Aruggo et al. also ref rence earlier work suggesting that E-selectin might be involved in tumor metastasis by mediating the adhesion of a colon carcinoma cell lin (HT-20) to activated endothelial cells. Platelet-leukocyte interactions are

believ d to be important in atheroscl rosis. Platelets might have a rol in r cruitm nt of monocytes into atherosclerotic plaques; the accumulation of monocytes is known t be one of the earliest d tectable vents during atherogenesis. Rupture of a fully developed plaque may not only lead to platelet deposition and activation and the promotion of thrombus formation, but also the early recruitment of neutrophils to an area of ischemia.

Another area of potential application is in the treatment of rheumatoid arthritis.

The criteria for assessing response to therapeutic modalities employing these peptides, and, hence, effective dosages of the peptides of this invention for treatment, are dictated by the specific condition and will generally follow standard medical practices. For example, the criteria for the effective dosage to prevent extension of myocardial infarction would be determined by one skilled in the art by looking at marker enzymes of myocardial necrosis in the plasma, by monitoring the electrocardiogram, vital signs, and clinical response. For treatment of acute respiratory distress syndrome, one would examine improvements in arterial oxygen, resolution of pulmonary infiltrates, and clinical improvement as measured by lessened dyspnea and tachypnea. For treatment of patients in shock (low blood pressure), the effective dosage would be based on the clinical response and specific measurements of function of vital organs such as th liver and kidney following restoration of blood pressure. Neurologic function would be monitored in patients with stroke. Specific tests are used to monitor the functioning of transplanted organs; for example, serum creatinine, urine flow, and serum electrolytes in patients undergoing kidney transplantation.

Diagnostic Reagents

The peptides can also be used for the detection of human disorders in which the ligands for the selectins might be defective. Such disorders would most likely be seen in patients with increased susceptibility to infections in which

leukocyt s might not be abl to bind to activat d platelets or endothelium. Cells to be tested, usually leukocytes, are collected by standard medically approved techniques and screened. Detection systems include ELISA procedures, binding of radiolabeled antibody to immobilized activated cells, flow cytometry, or other methods known to those skilled in the art. Inhibition of binding in the presence and absence of the lectin domain peptides can be used to detect defects or alterations in selectin binding. For selectins, such disorders would most likely be seen in patients with increased susceptibility to infections in which leukocytes would have defective binding to platelets and endothelium because of deficient leukocyte ligands for P-selectin.

The peptide is labeled radioactively, with a fluorescent tag, enzymatically, or with electron dense material such as gold for electron microscopy. The cells to be examined, usually leukocytes, are incubated with the labeled peptides and binding assessed by methods described above with antibodies to P-selectin, or by other methods known to those skilled in the art. If ligands for P-selectin are also found in the plasma, they can also be measured with standard ELISA or radioimmunoassay procedures, using labeled P-selectin-derived peptide instead of antibody as the detecting reagent.

The peptides can also be useful in *in vivo* imaging of concentrations of cells bearing selectin ligands. Cells expressing selectin ligands whose abnormally high local concentrations or presence within the body such as cancer cells, is indicative of a disorder can be imaged using labeled peptides. These labels may be either intrinsic or extrinsic to the structure of the specific selectin peptide and may include, but not be limited to high energy emitters such as ^{111}In or non-radioactive dense atoms to enhance x-ray contrast.

The following examples are pres nted to illustrate, not limit, the invention. In the examples and throughout the

specification, parts are by weight unless otherwise indicated.

EXAMPLE 1: Asparaginyl-argininyl-tyrosyl-thr nyl-aspartyl-leucyl-valyl-alanin -amid (SEQ ID NO: 3)

The peptide was prepared on an ABI Model 431A Peptide Synthesizer using Version 1.12 of the standard BOC software. 4-methyl benzhydrylamine resin (625 mg) was used in the synthesis. The final weight of the resin was 1.33 g.

The peptide was cleaved from the resin (1.33 g) using 13 mL of HF and 1.3 mL of anisole for 60 min at 0°C. The resin was washed with ether and the peptide extracted with 50% TFA in DCM to give 379 mg of crude peptide.

The crude peptide (299 mg) was purified on a Vydac C-18 column (15μ, 5 x 25 cm) eluting with a 10-50% gradient of 80% acetonitrile in 0.1% TFA over 120 min at a flow rate of 15 mL per min. Fractions were collected, analyzed by HPLC and pure fractions pooled and lyophilized to give 104 mg. Amino acid analysis: Ala 1.01 (1), Arg 0.98 (1), Asx 2.03 (2), Leu 1.04 (1), Thr 0.89 (1), Tyr 0.95 (1), Val 1.00 (1). FAB/MS: MH^+ 950.6

Example II: Asparaginyl-argininyl-tyrosyl-threonyl-aspartyl-leucyl-valyl-alanyl-isoleucine-amide (SEQ ID NO: 2)

The peptide was prepared on an ABI Model 431A Peptide Synthesizer using Version 1.12 of the standard BOC software. 4-methyl benzhydrylamine resin (625 mg) was used in the synthesis. The final weight of the resin was 1.42 g.

The peptide was cleaved from the resin (1.32 g) using 13 mL of HF and 1.3 mL of anisole for 60 min at 0°C. The resin was washed with 1:1 ether/methylene chloride and the peptide extracted with 50% TFA in methylene chloride to give 516 mg of crude peptide.

The crude peptide (300 mg) was purified on a Vydac C-18 column (15μ, 5 x 25 cm) eluting with a 25-50% gradient of 80% acetonitrile in 0.1% TFA over 120 min at a flow rate of 15 mL per min. Fractions were collected, analyzed by HPLC and pure fracti ns pooled and lyophilized to give 129 mg. Amino acid analysis: Ala 0.98 (1.0), Arg 1.04 (1.0), Asx

2.00 (2.0), Ile 0.94 (1.0), Leu 1.00 (1.0), Thr 0.93 (1.0), Tyr 0.95 (1.0), Val 1.09 (1.0). FAB/MS: MH^+ 1063

EXAMPLE III: Tyrosyl-threonyl-aspartyl-leucyl-valyl-alanyl-isoleucyl-amide (SEQ ID NO: 4)

The peptide was prepared on an ABI Model 431A Peptide Synthesizer using Version 1.12 of the standard BOC software. 4-methyl benzhydrylamine resin (0.625 g, 0.5 mmol) was used in the synthesis. The final weight of the resin was 1.05.

The peptide was cleaved from the resin (1.05) using 10 mL of HF and 1.5 mL anisole for 60 min at 0°C. The resin was washed with ether and the peptide extracted with 50% TFA in CH_2Cl_2 to give 317 mg of crude peptide.

The crude peptide (2 x 160) was purified on a Vydac C-18 column (15μ, 5 x 25 cm) eluting with a 10-50% gradient of 80% acetonitrile in 0.1% TFA over 120 min at a flow rate of 15 per min. Fractions were collected, analyzed by HPLC and pure fractions pooled and lyophilized to give 250 mg. Amino acid analysis: Ala 1.01 (1), Asx 1.04 (1), Ile 1.00 (1), Leu 1.04 (1), Thr 0.90 (1), Tyr 0.98 (1), Val 1.01 (1). FAB/MS: MH^+ 793.4

EXAMPLE IV: Arginyl-tyrosyl-threonyl-aspartyl-leucyl-valyl-alanyl-isoleucine-amide (SEQ ID NO: 1)

The peptide was prepared on an ABI Model 431A Peptide Synthesizer using Version 1.12 of the standard BOC software. 4-methyl benzhydrylamine resin (0.646 g, 0.5 mmol) was used in the synthesis. The final weight of the resin was 1.464 g.

The peptide was cleaved from the resin (1.358 g) using 14 mL of HF and 1.4 mL of anisole for 60 min at 0°C. The resin was washed with ether and the peptide extracted with a 1:1 solution of TFA:DCM to give 0.651 g of crude peptide.

The crude peptide (493 mg) was purified on a Vydac C-18 column (15μ, 5 x 25 cm) eluting with a 10-65% gradient of 80% acetonitril in 0.1% TFA over 120 min at a flow rate of 15 mL p r min. Fractions w re collected, analyzed by HPLC and pure fractions pooled and lyophilized to give 337 mg.

Amino acid analysis: Ala 1.03 (1), Arg 0.97 (1), Asx 1.04 (1), Ile 0.97 (1), Leu 1.04 (1), Thr 0.94 (1), Tyr 0.99 (1), Val 1.03 (1). FAB/MS: MH^+ 949

EXAMPLE V: Threonyl-aspartyl-leucyl-valyl-alanyl-isoleucyl-glutamine-amide (SEQ ID NO: 9)

The peptide was prepared on an ABI Model 431A Peptide Synthesizer using Version 1.12 of the standard BOC software. 4-methyl benzhydrylamine resin (0.668 g, 0.5 mmol) was used in the synthesis. The final weight of the resin was 1.12 g.

The peptide was cleaved from the resin (1.06 g) using 10 mL of HF and 1 mL of anisole for 60 min at 0°C. The resin was washed with ether and the peptide extracted with a 1:1 solution of TFA:DCM to give 0.615 g of crude peptide.

The crude peptide (400 mg) was purified on a Vydac C-18 column (15μ, 5.0 x 25 cm) by using two 200 mg injections eluting with a 0-65% gradient of 80% acetonitrile in 0.1% TFA over 120 min at a flow rate of 15 mL per min. Fractions were collected, analyzed by HPLC and pure fractions pooled and lyophilized to give 119 mg. Amino acid analysis: Ala 0.99 (1.00), Asx 1.01 (1.00), Glx 1.01 (1.00), Ile 0.96 (1.00), Leu 1.01 (1.00), Thr 0.90 (1.00), Val 1.03 (1.00). FAB/MS: MH^+ 758

EXAMPLE VI: Niflumyl-threonyl-aspartyl-leucyl-valyl-alanyl-isoleucyl-glutamine-amide (SEQ ID NO: 10)

The peptide was prepared on an ABI Model 431A Peptide Synthesizer using Version 1.12 of the standard BOC software. 4-methyl benzhydrylamine resin (0.625 mg, 0.5 mmol) was used in the synthesis. The final weight of the resin was 1.026 g.

The peptide was cleaved from the resin (0.941 g) using 10 mL of HF and 1.0 mL of anisole for 60 min at 0°C. The resin was washed with ether and the peptide extracted with a 1:1 solution of TFA:DCM to give 381 mg of crude peptide.

The crude peptide (381 mg) was purified on a Vydac C-18 column (15μ, 5 x 25 cm) eluting with a 25-75% gradient of 80% acetonitrile in 0.1% TFA over 120 min at a flow rate of

15 mL per min. Fractions were collected, analyzed by HPLC and pure fractions pooled and lyophilized to give 43 mg. Amino acid analysis: Ala 1.0 (1), Asx 1.0 (1), Glx 1.0 (1), Ile 0.97 (1), Leu 1.01 (1), Thr 0.65 (1), Val 1.0 (1). FAB/MS: MH^+ 1023

EXAMPLE VII: Thiobenzylcyclohexylacetyl-glycyl-aspartyl leucyl-valyl-alanyl-isoleucyl-glutamine-amide (SEQ ID NO: 11)

The peptide was prepared on an ABI Model 431A Peptide Synthesizer using Version 1.12 of the standard BOC software. 4-methyl benzhydrylamine resin (0.625 g, 0.5 mmol) was used in the synthesis. The final weight of the resin was 1.04 g.

The peptide was cleaved from the resin (1.00 g) using 10 mL of HF, 1.0 g of DL-DTT and 1 mL of anisole for 60 min at 0°C. The resin was washed with ether and the peptide extracted with TFA/DCM (1:1, v/v) (3 x 15 mL) to give 510 mg of crude peptide.

The crude peptide (500 mg) was purified on a Vydac C-18 column (15μ, 5 x 25 cm) eluting with a 30-85% gradient of B(80% acetonitrile) in 0.1% TFA over 120 min at a flow rate of 15 mL per min. Fractions were collected, analyzed by HPLC and pure fractions pooled and lyophilized to give 122 mg of white solid. Amino acid analysis: Ala 1.00 (1), Asx 1.01 (1), Glx 1.01 (1), Gly 1.01 (1), Ile 0.97 (1), Leu 1.00 (1), Val 1.00 (1). FAB/MS: MH^+ 960 (M.W. 960.21)

EXAMPLE VIII: Threonyl-aspartyl-leucyl-valyl-alanyl-isoleucyl-glutaminyl-asparaginyl-lysyl-asparaginyl-glutamic acid-amide (SEQ ID NO: 7)

The peptide was prepared on an ABI Model 431A Peptide Synthesizer using Version 1.12 of the standard BOC software. 4-methyl benzhydrylamine resin (0.625 g, 0.5 mmol) was used in the synthesis. The final weight of the resin was 1.590 g.

The peptide was cleaved from the resin (1.537 g) using 15 mL of HF and 1.5 mL of anisole for 60 min at 0°C. The resin was washed with ether and the peptide extracted with a 1:1 solution of TFA:DCM to give 1171 mg of crude peptide.

The crude peptide (935 mg) was purified on a Vydac C-18 column (15μ, 5 x 25 cm) eluting with a 10-50% gradient of 80% acetonitrile in 0.1% TFA over 120 min at a flow rate of 15 mL per min. Fractions were collected, analyzed by HPLC and pure fractions pooled and lyophilized to give 314 mg. Amino acid analysis: Ala 1.0 (1), Asx 3.06 (3), Glx 1.99 (2), Ile 0.97 (1), Leu 1.01 (1), Lys 1.01 (1), Thr 0.91 (1), Val 0.99 (1). FAB/MS: MH^+ 1243.6

EXAMPLE IX: Aspartyl-leucyl-valyl-alanyl-isoleucyl-glutamyl-asparaginyl-lysyl-asparaginyl-glutamic acid-amide (SEQ ID NO: 6)

The peptide was prepared on an ABI Model 431A Peptide Synthesizer using Version 1.12 of the standard BOC software. 4-methyl benzhydrylamine resin (0.625 g, 0.5 mmol) was used in the synthesis. The final weight of the resin was 1.410 g.

The peptide was cleaved from the resin (1.375 g) using 14 mL of HF and 1.4 mL of anisole for 60 min at 0°C. The resin was washed with ether and the peptide extracted with a 1:1 solution of TFA:DCM to give 789 mg of crude peptide.

The crude peptide (789 mg) was purified on a Vydac C-18 column (10μ, 2.2 x 25 cm) eluting with a 10-45% gradient of 80% acetonitrile in 0.1% TFA over 60 min at a flow rate of 10 mL per min. Fractions were collected, analyzed by HPLC and pure fractions pooled and lyophilized to give 294 mg. Amino acid analysis: Ala 1.01 (1), Asx 2.99 (3), Glx 2.02 (2), Ile 0.97 (1), Leu 1.01 (1), Lys 1.01 (1), Val 1.01 (1). FAB/MS: MH^+ 1142.4

EXAMPLE X: Leucyl-valyl-alanyl-isoleucyl-glutamyl-asparaginyl-lysyl-asparaginyl-glutamic acid-amide (SEQ ID NO: 8)

The peptide was prepared on an ABI Model 431A Peptide Synthesizer using Version 1.12 of the standard BOC software. 4-methyl benzhydrylamine resin (0.625 g, 0.5 mmol and Boc-Glu (cHex)) were used in the synthesis. The final weight of the resin was 1.25 g.

The peptide was cl aved from the r sin (1.2 g) using 12 mL of HF and 1.2 mL of anisol for 60 min at 0°C. The resin was washed with ether and the peptide extracted with TFA/DCM (1:1, v/v) (3 x 15 mL) to give 450 mg of crude peptide.

The crude peptide (450 mg) was purified on a Vydac C-18 column (15μ, 10 x 30 cm) eluting with a 0-45% gradient of 80% acetonitrile in 0.1% TFA over 60 min at a flow rate of 120 mL per min. Fractions were collected, analyzed by HPLC and pure fractions pooled and lyophilized to give 350 mg of white solid. Amino acid analysis: Ala 1.00 (1), Asx 2.01 (2), Glx 2.03 (2), Ile 0.98 (1), Leu 1.02 (1), Lys 0.99 (1), Val 0.99 (1). FAB/MS: MH^+ 1027.4

SEQUENCE LISTING

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(ix) TELECOMMUNICATION INFORMATION:
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(B) TELEFAX: 215-568-3439
(C) TELEX: 710-670-1334

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: p ptide

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 8
(D) OTHER INFORMATION: /label= Ile-NH2
/note= "The carboxy terminal amino acid, Ile, is amidated."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Arg Tyr Thr Asp Leu Val Ala Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 9
(D) OTHER INFORMATION: /label= Ile-NH2
/note= "The carboxy terminal amino acid, Ile, is amidated."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asn Arg Tyr Thr Asp Leu Val Ala Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 8
(D) OTHER INFORMATION: /label= Ala-NH2
/note= "The carboxy terminal amino acid, Ala, is amidated."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Asn Arg Tyr Thr Asp Leu Val Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION: /label= Ile-NH2
/note= "The carboxy terminal residue, Ile, is amidated."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Tyr Thr Asp Leu Val Ala Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION: /label= Val-NH2
/note= "The carboxy terminal residue, Val, is amidated."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asn Arg Tyr Thr Asp Leu Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 10
(D) OTHER INFORMATION: /label= Glu-NH2
/note= "The carboxy terminal residue, Glu, is amidated."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Asp Leu Val Ala Ile Gln Asn Lys Asn Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 11
(D) OTHER INFORMATION: /label= Glu-NH2
/note= "The carboxy terminal residue, Glu, is amidated."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Thr Asp Leu Val Ala Ile Gln Asn Lys Asn Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 9
(D) OTHER INFORMATION: /label= Glu-NH2
/note= "The carboxy terminal residue, Glu, is amidated."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Leu Val Ala Ile Gln Asn Lys Asn Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION: /label= Gln-NH2
/note= "The carboxy terminal amino acid, Gln, is amidated."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Thr Asp Leu Val Ala Ile Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1

(D) OTHER INFORMATION: /label= Niflumyl-Thr
/note= "A niflumyl group is attached to the amino terminal r sidue, Thr."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION: /label= Gln-NH2
/note= "The carboxy terminal residue, Gln, is amidated."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Xaa Asp Leu Val Ala Ile Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /label= modified-Gly
/note= "A thiobenzylcyclohexylacetyl group is attached to the amino terminal residue, Gly."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION: /label= Gln-NH2
/note= "The carboxy terminal residue, Gln, is amidated."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Xaa Asn Leu Val Ala Ile Xaa
1 5

WHAT IS CLAIMED IS:

1. Peptides of the formulas

$$R^1\text{-}W\text{-}A\text{-}B\text{-}C\text{-}D\text{-}X\text{-}R^2$$
(I)

and

$$R^1\text{-}Y\text{-}E\text{-}F\text{-}G\text{-}H\text{-}Z\text{-}R^2$$
(II)

or pharmaceutically acceptable salts thereof, where:

W and Y are an N-terminus amino acid linear sequence of from zero to 10 amino acids, and R^1 is a moiety attached to the terminal α amino group of W or Y, or the terminal α-amino group of A or E if W or Y respectively is zero;

X and Z are a C-terminus amino acid linear sequence of from zero to 10 amino acids, and R^2 is a moiety attached to the carboxyl carbon of X or Z or the carboxyl carbon of D or H if X or Z respectively is zero;

A is an amino acid selected from the group consisting of D- or L-tyrosine, D- or L-phenylalanine, D- or L-lysine, D- or L-glutamic acid, D- or L- arginine, D- or L-cysteine, D- or L-O-R^3-tyrosine, D- or L-N^α-R^3-tyrosine, D- or L-4-amino phenylalanine, D- or L-R^4-phenylalanine, D- or L-naphthylalanine, D- or L-pyridylalanine or D-L-tetrahydroisoquiniline carboxylic acid, where R^3 is lower alkyl or aryl, and R^4 is halogen;

B is an amino acid selected from the group consisting of D- or L-threonine, D- or L-lysine, D- or L-glutamic acid, D- or L-cysteine or glycine;

C is an amino acid selected from the group consisting of D- or L-aspartic acid, D-or L-histidine, D- or L-glutamic acid, D- or L-asparagine, D- or L-glutamine, D- or L-alanine, D- or L-phenylalanine, D- or L-lysine or glycine;

D is an amino acid selected from the group consisting of D- or L-leucine, D- or L-isoleucine, D- or L-alanine, D- or L-valine, D- or L-alleisoleucine, glycine, D- or L-

glutamic acid, D- or L-aspartic acid, D- or L-asparagine, D- or L-glutamine or D- or L-threonine;

provided that X is not J-K-L-Z′, where J is an amino acid selected from the group consisting of L-leucine, D-glutamic acid, D- or L-isoleucine, D- or L-alanine, D- or L-valine, glycine, D- or L-glutamic acid, D- or L-aspartic acid, D- or L-asparagine, and D- or L- glutamine; D- or L-threonine; and D- or L-alloisoleucine; K is D- or L-leucine, D- or L-isoleucine, D- or L-alanine, D- or L valine, glycine, D- or L-glutamic acid, D- or L-aspartic acid, D- or L-asparagine, D- or L-glutamine, D- or L threonine and D- or L-alloisoleucine; L is D- or L-glutamine, D- or L-glutamic acid, and D- or L-asparagine; and Z′ is a linear chain of from 0 to 7 amino acids;

E is an amino acid selected from the group consisting of D- or L-leucine, D- or L-isoleucine, D- or L-alanine, D- or L-valine, D- or L-alloisoleucine, glycine, D- or L-glutamic acid, D- or L-aspartic acid, D- or L-asparagine, D- or L-glutamine or D- or L-threonine;

F is an amino acid selected from the group consisting of D- or L-leucine, D- or L-isoleucine, D- or L-alanine, D- or L-valine, D- or L-alloisoleucine, glycine, D- or L-glutamic acid, D- or L-aspartic acid, D- or L-asparagine, D- or L-glutamine or D- or L-threonine;

G is an amino acid selected from the group consisting of D- or L-leucine, D- or L-isoleucine, D- or L-alanine, D- or L-valine, D- or L-alloisoleucine, glycine, D- or L-glutamic acid, D- or L-aspartic acid, D- or L-asparagine, D- or L-glutamine or D- or L-threonine;

H is an amino acid selected from the group consisting of glutamine, D-glutamine, glutamic acid, D-glutamic acid, asparagine or D-asparagine;

provided that Y is not M-N-P-Z″, where M is D- or L-tyrosine, D- or L-phenylalanine, D- or L-lysine, D- or L-glutamic acid, D- or L-arginine, D- or L- cysteine, D- or L-4-amino phenylalanine, D- or L-naphthylalanine, D- or 1-pyridylalanine, D- or L-tetrahydroisoquiniline, D- or L-O-R^3-

tyrosine, D- or L-N^2-R^3-tyrosine, D- or L-R^4-phenylalanine, where R^3 is lower alkyl or aryl, and R^4 is halog n; N is an amino acid s lected from th group c nsisting of D- or L-threonine, D- or L-lysine, D- or L-glutamine acid, D- or L-cysteine, and glycine; P is an amino acid selected from the group consisting of D- or L-aspartic acid, D- or L-histidine, D- or L-glutamic acid, D- or L-asparagine, D- or L-glutamine, D or L-alanine, D- or L-phenylalanine, D- or L-lysine and glycine; and Z″ is a linear amino acid chain of from 0 to 7 amino acids.

2. A peptide of claim 1 of the formula

$$R^1\text{-W-A-B-C-D-X-}R^2$$

(I)

or pharmaceutically acceptable salt thereof, where:

W is an N-terminus amino acid linear sequence of from zero to 10 amino acids, and R^1 is a moiety attached to the terminal α amino group of W;

X is a C-terminus amino acid linear sequence of from zero to 10 amino acids, and R^2 is a moiety attached to the carboxyl carbon of X;

A is an amino acid selected from the group consisting of D- or L-tyrosine, D- or L-phenylalanine, D- or L-lysine, D- or L-glutamic acid, D- or L- arginine, D- or L-cysteine, D- or L-O-R^3-tyrosine, D- or L-N^α-R^3-tyrosine, D- or L-4-amino phenylalanine, D- or L-R^4-phenylalanine, D- or L-naphthylalanine, D- or L-pyridylalanine or D-L-tetrahydroisoquiniline carboxylic acid, where R^3 is lower alkyl or aryl, and R^4 is halogen;

B is an amino acid selected from the group consisting of D- or L-threonine, D- or L-lysine, D- or L-glutamic acid, D- or L-cysteine or glycine;

C is an amino acid selected from the group consisting of D- or L-aspartic acid, D-or L-histidine, D- or L-glutamic acid, D- r L-asparagine, D- or L-glutamine, D- or L-alanine, D- or L-phenylalanine, D- or L-lysine or glycine;

D is an amino acid selected from the group consisting of D- or L-l ucine, D- or L-isoleucine, D- or L-alanine, D- or L-valine, D- or L-alleisoleucine, glycine, D- or L-glutamic acid, D- or L-aspartic acid, D- or L-asparagine, D- or L-glutamine or D- or L-threonine;

provided that X is not J-K-L-Z′, where J is an amino acid selected from the group consisting of L-leucine, D-glutamic acid, D- or L-isoleucine, D- or L-alanine, D- or L-valine, glycine, D- or L-glutamic acid, D- or L-aspartic acid, D- or L-asparagine, and D- or L- glutamine; D- or L-threonine; and D- or L-alloisoleucine; K is D- or L-leucine, D- or L-isoleucine, D- or L-alanine, D- or L valine, glycine, D- or L-glutamic acid, D- or L-aspartic acid, D- or L-asparagine, D- or L-glutamine, D- or L threonine and D- or L-alloisoleucine; L is D- or L-glutamine, D- or L-glutamic acid, and D- or L-asparagine; and Z′ is a linear chain of from 0 to 7 amino acids.

3. A peptide of claim 1 of the formula

$$R^1\text{-}Y\text{-}E\text{-}F\text{-}G\text{-}H\text{-}Z\text{-}R^2 \qquad \text{(II)}$$

or pharmaceutically acceptable salt thereof, where:

Y is an N-terminus amino acid linear sequence of from zero to 10 amino acids, and R^1 is a moiety attached to the terminal α amino group of Y;

Z is a C-terminus amino acid linear sequence of from zero to 10 amino acids, and R^2 is a moiety attached to the carboxyl carbon of Z;

E is an amino acid selected from the group consisting of D- or L-leucine, D- or L-isoleucine, D- or L-alanine, D- or L-valine, D- or L-alloisoleucine, glycine, D- or L-glutamic acid, D- or L-aspartic acid, D- or L-asparagine, D- or L-glutamine or D- or L-threonine;

F is an amino acid selected from the group consisting of D- or L-leucine, D- or L-isoleucine, D- or L-alanin , D- or L-valine, D- or L-alloisoleucine, glycine, D- or L-

glutamic acid, D- or L-aspartic acid, D- or L-asparagine, D- or L-glutamin or D- or L-thr onine;

G is an amino acid s lect d from the group consisting of D- r L-leucine, D- or L-isoleucine, D- or L-alanine, D- or L-valine, D- or L-alloisoleucine, glycine, D- or L-glutamic acid, D- or L-aspartic acid, D- or L-asparagine, D- or L-glutamine or D- or L-threonine;

H is an amino acid selected from the group consisting of glutamine, D-glutamine, glutamic acid, D-glutamic acid, asparagine or D-asparagine;

provided that Y is not M-N-P-Z″, where M is D- or L-tyrosine, D- or L-phenylalanine, D- or L-lysine, D- or L-glutamic acid, D- or L-arginine, D- or L- cysteine, D- or L-4-amino phenylalanine, D- or L-naphthylalanine, D- or 1-pyridylalanine, D- or L-tetrahydroisoquiniline, D- or L-O-R^3-tyrosine, D- or L-N^2-R^3-tyrosine, D- or L-R^4-phenylalanine, where R^3 is lower alkyl or aryl, and R^4 is halogen); N is an amino acid selected from the group consisting of D- or L-threonine, D- or L-lysine, D- or L-glutamine acid, D- or L-cysteine, and glycine; P is an amino acid selected from the group consisting of D- or L-aspartic acid, D- or L-histidine, D- or L-glutamic acid, D- or L-asparagine, D- or L-glutamine, D or L-alanine, D- or L-phenylalanine, D- or L-lysine and glycine; and Z″ is a linear amino acid chain of from 0 to 7 amino acids.

4. A peptide of Claim 1 wherein W is selected from the group consisting of Arg or Asn-Arg.

5. A peptide of Claim 1 wherein R^1 is Niflumyl (α,α,α-Trifluoro-m-toluidino)-nicotinyl).

6. A peptide of Claim 1 wherein Y is selected from group consisting of Asp-Leu or Thr-Asp-Leu.

7. A p ptid of Claim 1 wherein Z is slected from the group consisting of Asn, Asn-Lys, Asn-Lys-Asn, Asn-Lys-Asn-Glu.

8. A peptide of Claim 1 where R^1 is hydrogen, lower alkyl, aryl, formyl, alkanoyl, aroyl, alkyloxycarbonyl or arroyloxycarbonyl;

9. A peptide of Claim 1 where R^2 is OH, OR^3, signifying ester, where R^3 is lower alkyl or aryl or R^2 is NR^5R^6 where R^5 and R^6 are each selected independently from hydrogen, lower alkyl, aryl or cyclic alkyl.

10. A peptide of Claim 1 selected from the group consisting of peptides having this formula:

Arg-Tyr-Thr-Asp-Leu-Val-Ala-Ile-NH_2 (SEQ ID NO: 1)
Asn-Arg-Tyr-Thr-Asp-Leu-Val-Ala-Ile-NH_2 (SEQ ID NO: 2)
Asn-Arg-Tyr-Thr-Asp-Leu-Val-Ala-NH_2 (SEQ ID NO: 3)
Tyr-Thr-Asp-Leu-Val-Ala-Ile-NH_2 (SEQ ID NO: 4)
Asn-Arg-Tyr-Thr-Asp-Leu-Val-NH_2 (SEQ ID NO: 5)
Thr-Asp-Leu-Val-Ala-Ile-Gln-Asn-Lys-Asn-Glu-NH_2 (SEQ ID NO: 7)
Asp-Leu-Val-Ala-Ile-Gln-Asn-Lys-Asn-Glu-NH_2 (SEQ ID NO: 6)
Leu-Val-Ala-Ile-Gln-Asn-Lys-Asn-Glu-NH_2 (SEQ ID NO: 8)
Thr-Asp-Leu-Val-Ala-Ile-Gln-NH_2 (SEQ ID NO: 9)
Nif-Thr-Asp-Leu-Val-Ala-Ile-Gln-NH_2 (SEQ ID NO: 10)

11. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and an effective amount of one or more peptides of Claim 1.

12. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and an effective amount of one or more peptides of Claim 2.

13. A pharmac utical composition comprising a pharmac utically acceptabl carrier or diluent and an effectiv amount f one or more peptid s of Claim 3.

14. A method for inhibiting leukocyte adherence in a human patient comprising administering to said patient an effective quantity of a peptide of Claim 1.

15. A method for modifying binding of a selectin in a human patient comprising administering to said patient an effective quantity of a peptide of Claim 1.

16. A method of Claim 15 wherein said selectin is selected from the group consisting of P-selectin, E-selectin and L-selectin.

17. A method of treating a human patient in need of treatment for inflammation comprising administering to said patient an effective quantity of a peptide of Claim 1.

18. A method of treating a human patient in need of treatment for coagulation comprising administering to said patient an effective quantity of a peptide of Claim 1.

19. A method of treating a human patient for a condition selected from the group consisting of ischemia and reperfusion, bacterial sepsis and disseminated intravascular coagualation, adult respiratory distress syndrome, tumor metastasis, rheumatoid arthritis and atherosclerosis, comprising administering to said patient an effective quantity of a peptide of Claim 1.

20. A method of detecting defective selectin-binding ligands in a human patient comprising contacting cells from said patient with a labeled peptide of Claim 1, and assessing the binding f said label d peptide to said cells.

21. A method of Claim 20 wherein said c lls are leukocyt s.

22. A method of detecting high concentrations or elevated localized concentrations of selectin binding cells in a human patient comprising administering to said patient a labeled peptide from Claim 1 and assessing the binding of said labeled peptide to said cells.

23. A method of Claim 22 wherein said cells are leukocyte.

24. A method of Claim 22 wherein said cells are tumor cells.

25. A method of Claim 20 wherein said peptide is labeled with a moiety selected from the group consisting of radioactive tracers, fluorescent tags, enzymes, and electron-dense materials.

26. A method of Claim 21 wherein said peptide is labeled with a moiety selected from the group consisting of radioactive tracers, fluorescent tags, enzymes and electron-dense materials.

27. A method of preparing a peptide of Claim 1 comprising adding amino acids either singly or in pre-formed blocks of amino acids to an appropriately functionalized solid support.

28. The method of preparing a peptide of Claim 1 comprising adding amino acids either singly or in preformed blocks in solution or suspension by chemical ligation techniques.

29. The method of preparing a peptide of Claim 1 comprising adding amino acids either singly or in preformed

blocks in solution or suspension by enzymatic ligation techniqu s.

30. The method of preparing a peptide of Claim 1 comprising enzymatically by inserting nucleic acids encoding the peptide into an expression vector, expressing the DNA, and translating the DNA into the peptide.

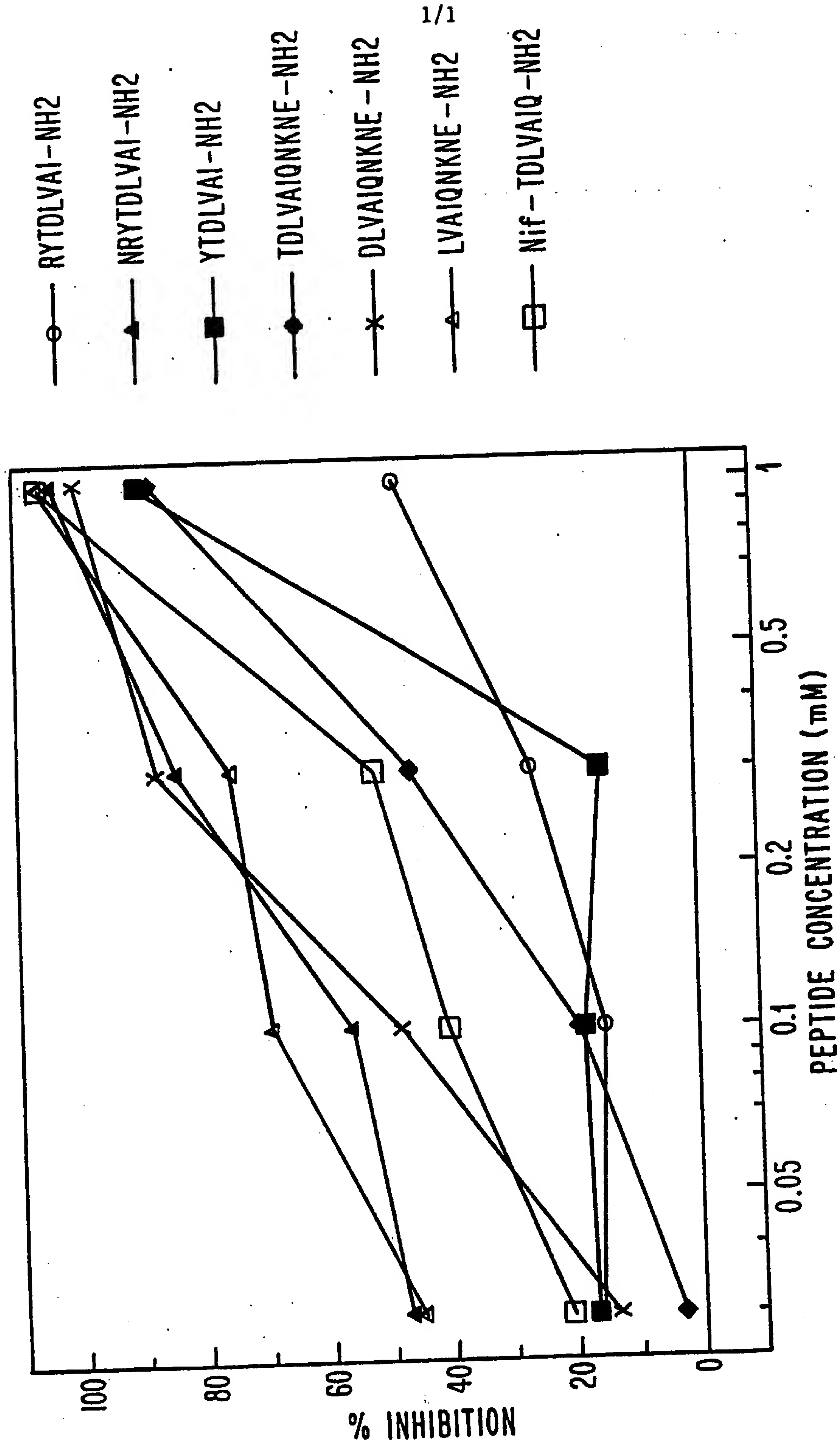
RYTDLVAI-NH2
NRYTDLVAI-NH2
YTDLVAI-NH2
TDLVAIQNKNE-NH2
DLVAIQNKNE-NH2
LVAIQNKNE-NH2
Nif-TDLVAIQ-NH2
% INHIBITION
0
20
40
60
80
100
0.05
0.1
0.2
0.5
1
PEPTIDE CONCENTRATION (mM)

Fig. 1

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :CO7K 07/06; A61K 37/00
US CL :514/15, 16; 530/329, 328, 327
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/15, 16; 530/329, 328, 327

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE, MEDLINE, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim N .
X,P	US, A, 5,198,424 (McEver) 30 March 1993, see entire document	1-30

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be part of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
30 July 1993

Date of mailing of the international search report
AUG 11 1993

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Form PCT/ISA/210 (second sheet)(July 1992)★